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(54) Title: NOVEL PHARMACEUTICAL COMPOSITION SUITABLE FOR GENE THERAPY

## (57) Abstract

There is provided according to the invention a pharmaceutical composition for gene therapy on cells which comprises: (a) nucleic acid encoding a therapeutic gene and a promoter; (b) a transfection vehicle; and (c) means to disrupt the function of the junctional complex in the cells together with methods of treatment using the same.

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Novel Pharmaceutical Composition Suitable for Gene Therapy

5 This invention relates to a novel pharmaceutical composition and its use in therapy. More particularly it relates to a novel composition for use in gene therapy.

10 In a variety of therapeutic situations it may be desirable to deliver therapeutic genes to patient's cells for the treatment of inherited (e.g. cystic fibrosis, muscular dystrophy) or acquired diseases (e.g. cancer and cardiovascular diseases). One major obstacle to the development of gene therapy is the 15 relatively poor efficiency of gene delivery.

It may be desirable to deliver therapeutic genes to the respiratory tract and 20 specifically the airway epithelium. This is particularly understood in relation to genetic diseases such as cystic fibrosis, but equally may be a suitable approach to the treatment of other diseases of the respiratory tract, such as chronic 25 obstructive pulmonary disease (COPD), asthma and lung fibrosis.

Traditional means of delivering genes to cells include transfection with viruses 20 (including adenoviruses, adeno-associated virus, retroviruses and lentiviruses) and with non-viral transfection agents (such as lipid/nucleic acid complexes). However it is a major challenge to achieve adequate levels of gene delivery in either case and low levels of transfection greatly increase the potential for 25 inflammatory or immune responses caused by the vehicle since high levels of vehicle have to be used. Thus, there is great need to find a way to increase levels of gene delivery in order to achieve the full benefits of these gene-based medicines.

We have now found a way to modify the cells so as to enhance transfection 30 efficiency particularly using viruses and non-viral delivery systems (e.g. using lipid complexes). It is known that penetration of the epithelium into the lateral space is controlled at the cell level by tight junctions that occur near the apical

surface. The formation and maintenance of these junctions are controlled by adherens or tight junctions. Desmosomes are also involved in the total junctional complex being important in the structural integrity of the epithelium and in controlling some aspects of epithelial phenotype, although they are believed to make a lesser contribution to barrier function. However surprisingly we have discovered that modification of the function of the junctional complex according to the invention in such cells allows access of vehicles for gene delivery to the basolateral membrane. However this approach is also believed to be applicable for vehicles applied to endothelial cells e.g. in the vasculature.

10 In specific embodiments we describe a way of enhancing gene delivery (e.g. to the airway epithelium) by disrupting the function of the adherens junction below the apical surface through disruption of E-cadherin function or by disrupting the function of the tight junction through disruption of occludin function.

15 The adherens junction is found in the junctional complex of polarised epithelial cells and is situated immediately basal to the tight junction. E-cadherin is a member of the calcium dependent adhesion molecule superfamily and is found in adherens junctions in epithelia, including those of the lung, gut and skin. It has a major role in controlling epithelial intercellular adhesion since it influences the formation of all epithelial intercellular junctions. Adhesion is mediated by interaction between extracellular domains of E-cadherin dimers on adjacent cells. In the adherens junction, cadherin dimers assemble in a zipper-like manner increasing the adhesive strength.

25 E-cadherin is a protein comprising an extracellular domain which comprises 5 sub-domains and a transmembrane domain. The zipper-like interaction of E-cadherin dimers on adjacent cells is believed to be mediated through the first (terminal) extracellular sub-domain (EC1) and the peptide sequence HAV is believed to be critical for this interaction. The amino acid sequence of human E-cadherin is shown in Figure A.

30

The tight junction is the most apical component of the junctional complex of polarised epithelial cells. This junction functions as a permeability barrier to the diffusion of solutes through the paracellular route. It also creates a boundary between the apical and basolateral domains of the plasma membrane that maintain cell polarity. Occludin is the integral membrane protein in the tight junction. The amino acid sequence predicts four transmembrane domains with two extracellular loops (Loop I and Loop II) and these loops are thought to create the tight junction seal. A synthetic peptide corresponding to Loop II of the extracellular domain (the loop closest to the C-terminal tail) of occludin has been shown to perturb the tight junction permeability barrier causing loss of tight junction function (Wong and Gumbiner BM, 1997). The amino acid sequence of human occludin is shown in Figure B.

The junctional complex of endothelial cells contains occludin in the tight junction (as with epithelial cells) however in the adherens junction it contains the protein VE-cadherin, which is a structurally distinct relative of E-cadherin (Brevario et al, 1995).

E-cadherin recognition has been described in Lutz et al., (1995) where extracellular peptide sequences were identified using anti-E-cadherin antibodies. Complications concerning gene therapy caused by the fact that poorly differentiated airway epithelial cells are easily transfectable by liposome-DNA complexes, whereas more differentiated central cells were not, was described in Matsui et al., (1997). Croyle et al., (1998) have described the importance of  $\alpha_v\beta_3$  and  $\alpha_6\beta_1$  integrins in adenoviral internalisation in the intestinal epithelium. Using fluorescence microscopy, Chu et al., (1999) demonstrated that polarised apical membranes (i.e. tight junctions) of airway epithelial cells are resistant to transfection by cationic lipid : plasmid DNA complexes. Furthermore, Walters et al., (1999) have shown that upon transient disruption of the integrity of tight junctions in airway epithelial cells, apically applied adenovirus gained access to the basolateral surface and enhanced gene transfer.

Thus, according to a first aspect of the invention, we provide a pharmaceutical composition for gene therapy on cells which comprises:

- (a) nucleic acid encoding a therapeutic gene and a promoter;
- (b) a transfection vehicle; and
- 5 (c) means to disrupt the function of the junctional complex in the cells.

Coexpression of a secreted marker from the same nucleic acid vector either using the same promoter as used for the therapeutic gene of interest or a different promoter might allow simple assessment of gene expression by 10 measuring the marker in, for example, induced sputum, serum or urine. This would be helpful in determining the efficiency of gene delivery and longevity of gene expression and help determine when the next session of gene therapy was required. Therefore, in a composition according to the invention, preferably the nucleic acid (element (a)) will also encode a secreted marker.

15 Examples of therapeutic genes which may desirably be administered in this way include the genes for cystic fibrosis (e.g. as described in Engelhardt et al, 1993). In addition delivery of genes controlling inflammation (e.g. IL-10) or fibrosis (e.g. LAP (Latency Associated Peptide, which binds to TGF $\beta$  rendering it inactive)) 20 could be useful for treatment of inflammatory or fibrotic lung diseases.

Other genes of interest which may be administered in this way include:

genes for induction of angiogenesis (e.g. VEGF or FGF) for the treatment of 25 peripheral vascular disease and coronary arterial disease;

genes for induction of cell ablation (e.g. HSV tk when coadministered with ganciclovir) for the treatment of restenosis;

low density lipoprotein receptor (LDL $r$ ), ApoE or ApoA1 for treatment of 30 hypercholesterolaemia;

genes for induction of cell ablation (e.g. thymidine kinase when coadministered with ganciclovir or cytosine deaminase when co-administered with 5-fluorocytosine) for treatment of cancers;

tumour suppressor genes (e.g. p53) or immunomodulators (e.g. IL-2, IL-12, gamma IFN, GM-CDF or G-CSF) for the treatment of cancers.

5 Examples of suitable promoters include CMV promoter, human surfactant protein C promoter, cytokeratin 18 promoter and Clara cell 10 promoter. The CMV promoter is preferred.

10 Transfection vehicles may be viral or non-viral. Examples of transfection vehicles include respiratory viruses such as adenovirus. Recombinant deficient adenoviruses have been used for gene therapy and have the advantage that they will infect non-dividing cells. This is important when the rate of cell division in the target tissue is low, as is the case in airway epithelium. The disadvantage of this approach is that gene expression is transient and thus repeated exposure to the treatment is required. Concerns have been raised over the inflammatory 15 response induced by adenoviral exposure, although recent work has suggested that further viral genome deletion may reduce this effect. Moreover, recombination with endogenous virus is an inherent potential risk. Retroviruses have also been considered for gene therapy, these having the advantage that stable integration of the exogenous gene into the host genome would mean that 20 this treatment, if successful, would require less frequent exposure. However, there are risks from insertional mutagenesis in this approach. Moreover, retroviruses require host cell division for successful integration; if cell replication is low, then efficiency of gene delivery will also be low. However lentivirus vectors will integrate in both dividing and non-dividing cells. Adeno-associated 25 virus has also been used as a vehicle for gene delivery and has the advantage of potentially giving long term gene expression and that no known disease in humans has been noted, so recombination with endogenous virus seems less problematic (Teramoto et al, 1998).

30 Adenovirus is of most particular interest as a transfection vehicle. Adeno-associated virus is also of particular interest. Retroviruses are also of particular interest. Lentivirus vectors are also of interest.

For viral transfection vehicles, the nucleic acid encoding the therapeutic gene and promoter may be inserted into an appropriate viral vector (e.g. a viral plasmid).

5

Other examples of transfection vehicles include non-viral delivery systems such as cationic lipids, liposomes or cationic polymers like polyethyleneimine (PEI) or polylysine which are generally capable of forming a complex with nucleic acid. These cationic agents are complexed with nucleic acid which encodes the therapeutic gene and promoter. Other agents that enhance gene delivery by increasing endosomal release (e.g. fusogenic lipids or peptides) or targeting ligands (e.g. peptides or antibodies) that increase binding to specific cells may be incorporated into these complexes. The advantage of these vehicles over viral vectors for gene delivery is that they should cause less or no inflammatory response in the host and no long-term immune response would be expected. This means that it should be safe to use them repeatedly, which would be highly desirable if gene expression was only transient. However, generally, they have only a low transfection efficiency.

20

Cationic lipids are preferred, such as those described by Lee et al (1996). Particular examples include cationic lipid GL-67 as described by Lee, 3 $\beta$  [ N-(N' N'- dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol), N-[1-(2,3-Dioleyloxy)propyl]-N,N,N-trimethylammonium methyl sulfate (DOTAP), ethyl dimirystoyl phosphatidyl choline (EDMPC) and 1,2-dimiristyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide (DMRIE). These cationic lipids are often mixed with fusogenic neutral lipids, such as dioleoylphosphatidylethanolamine (DOPE) and dioleoylphosphatidylcholine (DOPC) or cholesterol. Avanti Polar Lipids (Alabaster, AL) is a supplier of such lipids. Combinations of lipids (e.g. GL-67: DOPE 2:1, DMRIE:cholesterol 1:1, EDMPC: cholesterol 1:1 and DC-Chol: DOPE 6:4) may be used.

As mentioned above, other cationic agents include polyethylenimine (PEI) or polylysine which can condense nucleic acid and enhance uptake by cells.

5 Peptides may also be incorporated into the complex to mediate endosomal release (e.g. HA2 peptides from influenza virus). Similarly, targeting ligands or nuclear localisation signal peptides may be incorporated into the complexes. These may be linked covalently to the cationic lipid or polymer or linked to a cationic moiety so that they are able to bind DNA independently.

10 When the transfection vehicle is a retrovirus, the nucleic acid encoding the therapeutic gene (and promoter) will preferably be RNA. For other applications it will preferably be DNA.

15 The function of the junctional complex will be disrupted, for example, in epithelial cells or endothelial cells. Typically the epithelial or endothelial cells will be polarised.

20 The function of the junctional complex may be disrupted by disrupting the function of the adherens junction, the tight junction or desmosomes. However disruption of the adherens junction or tight junction will be preferred.

25 In one principal embodiment of the invention epithelial adherens junction function is disrupted by disrupting E-cadherin function.

30 In preferred embodiments of the invention the means to disrupt E-cadherin function in the adherens junction epithelium will specifically disrupt E-cadherin function in the adherens junction of the epithelium. Thus non specific disruption (e.g. through agents which chelate metal ions such as calcium and magnesium e.g. EDTA and EGTA) is preferably avoided. This approach is advantageous since it is more likely to achieve the benefit of improved transfection efficiency without causing undesirable side effects. Divalent metal ions, for example, have a multitude of roles in cells. Thus compositions according to the invention

will preferably not contain non-specific disrupters of E-cadherin function (or affinity) such as chelating agents e.g. EDTA and EGTA.

5 One means to specifically disrupt E-cadherin function in the adherens junction of the epithelium is by use of anti-E-cadherin antibodies. Typically the antibodies will be monoclonal antibodies raised in mouse against human E-cadherin. Methods of preparing monoclonal antibodies comprising fusing antibody producing cells with immortal cells to produce hybridomas will be well known to a person skilled in the art.

10 Certain anti-E-cadherin antibodies are described in EP821060 (Becker et al) and WO92/17608 (Behrens et al) both of which documents are hereby incorporated by reference.

15 Another means to specifically disrupt E-cadherin function in the adherens junction of the epithelium is by use of peptides capable of preventing interaction of E-cadherin molecules in the adherens junction. Examples of such peptides include peptide fragments from the E-cadherin extracellular domain (Lutz et al, 1995). Peptides will generally have a length of at least 10 - 20 amino acids, the larger peptides often having higher affinities for E-cadherin. In the context of 20 gene delivery to the lungs, there may be advantages in having larger peptides, possibly the entire E-cadherin extracellular domain (so-called "soluble E-cadherin"), so that system exposure is reduced. Cyclic peptides for disruption of E-cadherin function have also been described (WO9802452, Univ. McGill).

25 In an alternative embodiment of the invention endothelial adherens junction function is disrupted by disrupting VE-cadherin function. VE-cadherin function may be disrupted specifically using anti-VE-cadherin antibodies which may be prepared as described above. Peptides which prevent interaction of VE- 30 cadherin in the endothelial adherens junction may also be used.

In a second principal embodiment, tight junction function will be disrupted by disrupting occludin function. In preferred embodiments occludin function is disrupted specifically for the reasons given above.

5 One means to specifically disrupt occludin function in the tight junction is by use of anti-occludin antibodies. Typically the antibodies will be monoclonal antibodies raised in mouse against human occludin. As discussed above, methods of preparing monoclonal antibodies will be well known to a person skilled in the art.

10 Another means to specifically disrupt occludin function in the tight junction is by use of peptides capable of preventing interaction of occludin molecules in the tight junction. Examples of occludin function disrupting peptides include a synthetic peptide corresponding to Loop II of the extracellular domain (the loop closest to the C-terminal tail). This peptide has been shown to perturb the tight junction permeability barrier causing loss of tight junction function (Wong V. & Gumbiner BM, 1997.). Occludin function disrupting peptides will generally have a length of at least 10 - 20 amino acids, the larger peptides often having higher affinities for occludin.

15

20 Two example peptides are:

Chicken occludin peptide (OCC2) from Wong and Gumbiner, 1997:

GACQVYDPYMSGNYPAQSLMYQNLQYLVSGIHTYPECSYATQSY

25 human occludin peptide from homology with chicken sequence (not published)

GVNPQAQMSSGYYYSPLLAMCSQAYGSTYLNQYIHYCTVDPQE

30 Tight junction function may also be disrupted by disruption of claudins function. Claudins are a family of proteins also present in tight junctions which, at least in some circumstances, appear able to make a tight junction seal in the absence of functional occludin (Furuse et al, 1998; Morita et al, 1999). Claudins function may be disrupted e.g. by use of anti-claudins antibodies.

Tight junction function may also be disrupted by use of chitosan.

5 Preferably antibodies will be humanised. Humanised antibodies may be prepared as described by Reichman et al (1998).

10 We also provide a method of enhancing efficiency of gene delivery to cells in a pharmaceutical composition for gene therapy comprising nucleic acid encoding a therapeutic gene and a promoter and a transfection vehicle which comprises including means to disrupt the function of the junctional complex in the cells.

We also provide a method of performing gene therapy on cells of a patient which comprises administering a composition which comprises:

15 (a) nucleic acid encoding a therapeutic gene and a promoter;  
(b) a transfection vehicle; and  
(c) means to disrupt function of the junctional complex of the cells.

20 We also provide use of means to disrupt function of the junctional complex of cells together with nucleic acid encoding a therapeutic gene and a promoter and a transfection vehicle in the manufacture of a pharmaceutical composition for gene therapy.

25 We have also discovered that further improvements in transfection efficiency in the case of viral transfection vehicles might be gained by enhancing certain integrin function in the airway epithelium. Specifically we have shown that  $\alpha_v\beta_5$  integrin is important in adenoviral infection and that enhancing the expression or function of this integrin improves infection efficiency.

30 Cell surface integrins have at least two principal functions, namely: (i) binding to substrates e.g. basement membrane and integrins on other cells and (ii) mediating internalisation of exogenous substances.

Surprisingly we have also discovered that cell surface integrin function may be enhanced by use of peptides containing the motif RGE ("RGE peptides"). Although it is known that peptides containing the motif RGD ("RGD peptides") are capable of binding cell surface integrins (since, for example, the penton base of the adenovirus contains a protein containing the RGD motif which is believed to bind integrins and facilitate infectivity), RGE peptides would not be expected to bind. However, without being limited by theory, we believe that weak binding of RGE peptides to cell surface integrins may increase their turnover/recycling and thereby enhance the internalisation function.

We have also found that transfection may be enhanced by enhancing the affinity of binding of the viral transfection vehicle to cell surface integrins.

Thus as a second aspect of the invention we provide a pharmaceutical composition for gene therapy which comprises:

- nucleic acid encoding a therapeutic gene and a promoter;
- a viral transfection vehicle;
- means to disrupt function of the junctional complex in cells ; and
- means to enhance cell surface integrin function or affinity of binding of the viral transfection vehicle to cell surface integrins.

Examples of means to enhance cell surface integrin function include RGE peptides as discussed above. Examples of means to enhance affinity of binding of the viral transfection vehicle include RGD and RGE peptides.

Cell surface integrins include  $\alpha_v\beta_3$ ,  $\alpha_v\beta_5$ ,  $\alpha_4\beta_1$ ,  $\alpha_{IIIb}\beta_3$ ,  $\alpha_5\beta_1$  and  $\alpha_9\beta_1$  integrins. The  $\alpha_v\beta_3$  integrin is of principal interest.  $\alpha_v\beta_5$  integrin is also of particular interest.  $\alpha_9\beta_1$  integrin is also of particular interest.

RGD sequences may be cloned into the adenovirus fibre protein that normally binds the CAR (coxsackie and adenovirus) receptor (Wickham et al. 1997). These sequences would allow both the fibre and penton base to bind  $\alpha_v\beta_5$  and

$\alpha_v\beta_3$  integrins on the cell surface. Alternatively, a bispecific ligand can be used to re-target adenovirus fibre to integrins on the cell surface (Wickham et al. 1996).

5 Also, peptides with specificity for different integrins can be used in place of the RGD peptides. For example, the EILDVPST sequence from vitronectin will bind specifically to  $\alpha_4\beta_1$  expressed on cells such as lymphocytes and macrophages, while the  $\beta_3$ -specific RGD motif (e.g. SFGRGDIRN) has been reported to interact with high affinity to the integrins  $\alpha_v\beta_3$  and  $\alpha_{IIIb}\beta_3$ , but shows no binding to  $\alpha_v\beta_5$  or 10  $\alpha_5\beta_1$  integrins.(Barbas et al. 1993).

Peptides containing the motif IDG (e.g. PLAEIDGIELTY) are also believed to bind  $\alpha_9\beta_1$  integrin.

15 The means to used enhance affinity of binding of the viral transfection vehicle to cell surface integrins should not inhibit integrin function. Thus RGD peptides should be bound to the viral transfection vehicle so as to aid targeting to the integrins and so as not to compete with the vehicle for binding to integrins.

20 Short peptide sequences would be preferred e.g. 5-15 amino acid, more preferably 5-12 amino acids, more preferably 5-6 amino acids. An example of an RGE peptide has the sequence GRGES. Another example has sequence GGCRGEMFGCGG.

25 The viral transfection vehicle is preferably an adenoviral transfection vehicle or an adeno-associated virus transfection vehicle (more preferably the former). It may also preferably be a retroviral or lentiviral transfection vehicle.

30 Further, we have also found that cellular uptake of non-viral delivery system based compositions (e.g. comprising lipid transfection vehicles) may be improved by enhancing the function of cell surface integrins or enhancement of their affinity for the non-viral transfection vehicle.

Thus as a third aspect of the invention we provide a pharmaceutical composition for gene therapy which comprises:

- (a) nucleic acid encoding a therapeutic gene and a promoter;
- 5 (b) a non-viral transfection vehicle;
- (c) means to disrupt the function of the junctional complex in cells ; and
- (d) means to enhance cell surface integrin function or affinity of binding of the non-viral transfection vehicle to cell surface integrins.

10 Examples of means to enhance cell surface integrin function include RGE peptides as discussed above. Examples of means to enhance affinity of binding of the non-viral transfection vehicle include RGD and RGE peptides.

15 Short peptide sequences would be preferred e.g. 5-15 amino acid, more preferably 5-12 amino acids, more preferably 5-6 amino acids. An example of an RGD peptide has the sequence GRGDSP. Another example has sequence GGCRGDMFGCGG.

20 The peptide may in addition have a cationic tail capable of causing binding of the peptide to the nucleic acid which is desirable since it assists targeting of the lipid/DNAcomplex to the cell surface integrins.

25 Suitable cationic tails include a poly-K (lysine) C-terminal tail e.g. a tail of 5-60, say 16 K residues. Presence of this tail is desirable since it permits the peptide to bind to the nucleic acid thereby assisting targeting of the non-viral transfection vehicle to the cell surface integrins.

Example RGD and RGE peptides with poly K tail are GGCRGDMFGCGG-(K)<sub>16</sub> (Hart et al, 1995; 1997) and GGCRGEMFGCGG-(K)<sub>16</sub>.

30 PolyR (arginine) tails may also be suitable. Alternatively a polyethyleneimine (PEI) tail may be used.

The peptide may, alternatively, be covalently bound to the lipid e.g. through phosphatidylethanolamine which achieves the same purpose of targeting the lipid/DNA complex to the cell surface integrins.

5

The benefit of the RGD and RGE peptides in enhancing transfection mediated through non-viral transfection vehicles is surprising since, as discussed above, research into RGD peptides has been stimulated by their origin in adenoviral penton protein and cell adhesion proteins such as fibronectin which are physically unrelated systems.

10

The non-viral transfection vehicle is preferably a lipid transfection vehicle, especially a cationic lipid.

15

Small molecules that mimic RGD and RGE peptides or are otherwise effective in enhancing cell surface integrin function or affinity of the transfection vehicle for cell surface integrins are also envisaged as an aspect of the invention.

20

We also provide a method of enhancing efficiency of gene delivery to cells in a pharmaceutical composition for gene therapy comprising nucleic acid encoding a therapeutic gene and a promoter, a viral transfection vehicle and means to disrupt function of the junctional complex in the cells which comprises including means to enhance cell surface integrin function or the affinity of binding of the viral transfection vehicle to cell surface integrins.

25

We also provide a method of enhancing efficiency of gene delivery to cells in a pharmaceutical composition for gene therapy comprising nucleic acid encoding a therapeutic gene and a promoter, a non-viral transfection and means to disrupt the function of the junctional complex of the cells which comprises including means to enhance cell surface integrin function or affinity of binding of the non-viral transfection vehicle to cell surface integrins.

30

We also provide a method of performing gene therapy on cells of a patient which comprises administering a composition which comprises:

- (a) nucleic acid encoding a therapeutic gene and a promoter;
- (b) a viral transfection vehicle;
- 5 (c) means to disrupt the function of the junctional complex of the cells; and
- (d) means to enhance cell surface integrin function or affinity of binding of the viral transfection vehicle to cell surface integrins.

10 We also provide a method of performing gene therapy on cells of a patient which comprises administering a composition which comprises:

- (a) nucleic acid encoding a therapeutic gene and a promoter;
- (b) a non-viral transfection vehicle;
- (c) means to disrupt the function of the junctional complex of the cells; and
- 15 (d) means to enhance cell surface integrin function or affinity of binding of the non-viral transfection vehicle to cell surface integrins.

20 We also provide use of means to enhance cell surface integrin function or affinity of binding of a viral transfection vehicle to cell surface integrins together with nucleic acid encoding a therapeutic gene and a promoter, a viral transfection vehicle and means to disrupt the function of the junctional complex of cells in the manufacture of a pharmaceutical composition for gene therapy.

25 We also provide use of means to enhance cell surface integrin function or affinity of binding of a non-viral transfection vehicle to cell surface integrins together with nucleic acid encoding a therapeutic gene and a promoter, a non-viral transfection vehicle and means to disrupt the function of the junctional complex of cells in the manufacture of a pharmaceutical composition for gene therapy.

30 As a less preferred aspect of the invention we also provide a pharmaceutical composition for gene therapy which comprises:

- (a) nucleic acid encoding a therapeutic gene and a promoter;
- (b) a viral transfection vehicle; and

(c) means to enhance cell surface integrin function or the affinity of binding of the viral transfection vehicle to cell surface integrins.

5 We also provide a pharmaceutical composition for gene therapy which comprises:

- (a) nucleic acid encoding a therapeutic gene and a promoter;
- (b) a non-viral transfection vehicle; and
- (c) means to enhance cell surface integrin function or the affinity of binding of the non-viral transfection vehicle to cell surface integrins.

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Formulations containing lipids may be prepared by solubilising the lipids e.g. in chloroform and then drying them down to a thin film under a stream of nitrogen. Residual solvent may be removed under high vacuum.

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Lipid vesicles may be prepared by hydrating lipid vesicles in distilled water.

20

Lipid:nucleic acid complexes may be prepared by preparing the vesicles at excess concentration (typically 20 times the desired final concentration) and mixing them with an equal volume of concentrated nucleic acid (typically 20 times the desired final concentration) in distilled water or Tris buffer pH 7.0-8.5. After incubation (typically 10-60 minutes at room temperature) the complexes may be diluted e.g. 10 fold in a buffer (e.g. Opti-MEM buffer, GIBCO-BRL, Gaithersburg, MD or, more preferably, PBS (phosphate buffered saline) or Tris buffer pH 7.0-8.5). The average size (diameter) of the lipid:DNA complexes formed in this way is approximately 25-500 nm.

25

Other non-viral delivery systems incorporating cationic polymers such as PEI, chitosan or polylysine can be produced by mixing the cationic agent (typically 20 times the desired final concentration) with an equal volume of concentrated nucleic acid (typically 20 times the desired final concentration) in distilled water or Tris buffer pH 7.0-8.5. After incubation (typically 10-60 minutes at room temperature) the complexes may be diluted e.g. 10 fold in a buffer (e.g. Opti-MEM buffer, GIBCO-BRL, Gaithersburg, MD or PBS or Tris buffer pH 7.0-8.5).

The average size (diameter) of the complexes formed in this way is approximately 25-500 nm.

Adenovirus vectors are produced by cloning the therapeutic gene and eukaryotic promoter (tissue-specific or constitutive) into a transfer vector. These are generally cloned in to replace the E1 region. The sequences in the transfer vector are then transferred into the viral genome by recombination with a helper plasmid either in *E. coli* or in eukaryotic cells that support viral replication (e.g. HEK293 or HER911 cells). If the recombination is carried out in *E. coli* the infectious viral genome is transfected into the adenovirus producer cells (e.g. 293 or 911). The transfection of the adenovirus producer cells with the transfer vector and the subsequent recombination or with infectious viral DNA leads to production of replication defective virus from these cells. This virus is propagated in bioreactors and collected.

Compositions according to the invention comprising a viral transfection vehicle may then be prepared by combining the virus particles with the means to specifically disrupt function of the junctional complex. Means to enhance cell surface integrin function or affinity for integrins (e.g. RGD peptides) may also be included as desired. The composition will preferably be provided as an aqueous suspension.

Compositions according to the invention comprising a non-viral transfection vehicle may be prepared by complexing viral coat proteins with nucleic acid and combining these virus-like particles with the means to specifically disrupt function of the junctional complex. The nucleic acid may be complexed via a cationic sequence (e.g. polylysine). Means to enhance cell surface integrin function or affinity for integrins (e.g. RGD peptides) may also be included as desired. The composition will preferably be provided as an aqueous suspension.

Compositions according to the invention comprising a non-viral transfection vehicle may be prepared by incubating nucleic acid and the cationic lipid or polymer as described above and combining this complex with the means to specifically disrupt function of the junctional complex. Means to enhance cell 5 surface integrin function or affinity may also be included as desired. When the means to enhance cell surface integrin function or affinity is capable of binding nucleic acid (e.g. RGD-polylysine) it may be incubated with the DNA before or after combination with cationic lipid or polymer. The composition will preferably be provided as an aqueous suspension.

10 Cells of the airway epithelium are a target of particular interest. Compositions destined for the airway epithelium may be administered to a patient to the lung by nebulisation or other aerosolisation over a period of minutes or up to three hours. Compositions destined for the cells of tumours may be administered by 15 intratumoral injection or intravenous (i.v.) injection. Compositions destined for the cells of the vasculature may be administered by iv injection. I.v. injections may be by continuous infusion or bolus injection.

20 Where reference is made to E-cadherin, occludin and claudins it will be understood that reference is intended to the human proteins unless otherwise stated.

#### Examples

#### Materials and Methods

25 A human bronchial epithelial cell line, 16HBE14o-, was used in these studies. Cells were seeded into chamber slides or 96 well plates at  $1 \times 10^4$  cells / well, or Transwell membranes (Costar) at  $4 \times 10^4$  cells / membrane, in growth medium consisting of Eagles MEM with Earle's salts, and supplemented with 5% FCS, 2mM glutamine and antibiotics.

30 Transepithelial electrical resistance (TER) was measured using a Millipore ERS system. Adenovirus RAd35 was used for the infection studies. This is a

replication deficient, recombinant virus containing a lacZ reporter gene encoding β-galactosidase and was a kind gift from Gavin Wilkinson (Wilkinson et al, 1992). Cells on membranes exposed for 24hrs to either control IgG2a or SHE78-7, were incubated with virus for 6hrs. For the RGE peptide studies, antibodies 5 were added to the cells 15min prior to virus incubation. Cultures were then washed in PBS and either extracted for measurement of β-galactosidase activity using a Promega β-galactosidase assay kit, or were fixed in 2% formaldehyde, 0.2% glutaraldehyde, then stained with X-gal.

10 SHE78-7 is an anti-human E-cadherin (extracellular domain) monoclonal antibody obtained from mouse, which was obtained commercially (Takara Biomedicals). ECCD-2 and DECMA-1 are anti-mouse E-cadherin monoclonal antibody obtained from rat, which were obtained commercially (Takara Biomedicals and 15 Sigma).

20 Lipid DOTAP was obtained from Boehringer Mannheim. Lipid EDMPC was obtained from Sigma however references to lipid "EDMPC" in the examples and figures are to EDMPC: cholesterol 1:1. Lipid DMRIE-C was obtained from Life Tech. References to lipid "DC-Chol" in the examples and figures are to DC-Chol/DOPE 6:4 which was kindly provided by Bill Colledge (Cambridge).

25 Anti-integrin antibodies (anti-human integrin antibodies obtained from mouse) were obtained commercially. Anti-α v β3 and α v β5 antibodies (Chemicon International) bind the dimer; anti- α v antibodies (Immunotech; clones cat nos 1603 and 0770) bind just the α v subunit only.

30 GRGESP and GRGDSP peptides were obtained from GIBCO BRL. GGCRGEMFGCGG-(K)<sub>16</sub> and GGCRGDMFGCGG-(K)<sub>16</sub> peptides were prepared by known methods.

The reference to "RGE peptide" or "RGD peptide" for lipid transfection studies is to the peptides GGCRGEMFGCGG-(K)<sub>16</sub> and GGCRGDMFGCGG-(K)<sub>16</sub> respectively. The reference to "RGE peptide" for the viral infection/transfection studies is to the peptide GRGESP.

5

**Brief description of Figures:**

Figure A: Peptide and nucleotide sequence of human E-cadherin

Figure B: Peptide sequence of human occludin

10 Figure 1: RAd 35 adenovirus containing LacZ infection of 16HBE14o- cells incubated with control or SHE78-7 for 24 hours: colony disruption

Figure 2: Effect of SHE78-7 on transepithelial resistance in 16HBE14o- cells.

Figure 3: RAd35 adenovirus infection of 16HBE14o- cells incubated with IgG2a or SHE78-7 antibody for 24 hours

15 Figure 4: RAd35 adenovirus infection of 16HBE14o- cells incubated with IgG2a or SHE78-7 antibody for 24 hours (dose response)

Figure 5: Effect of anti-integrin antibodies on Rad35 infectivity of SH78-7 treated 16HBE14o-cells.

Figure 6: Effect of ECCD-2 and DECMA-1 antibodies on transepithelial resistance of mouse tracheal epithelial cells

20 Figure 7: Effect of ECCD-2 and DECMA-1 on RAd35 infectivity of mouse primary tracheal epithelial cells.

Figure 8a,b: Enhancement of lipid mediated gene delivery to 16HBE14o- cells using SDE78-7

25 Figure 9a,b: Enhancement of RAd35 adenovirus transfection efficiency using RGE peptide and SHE78-7 antibody.

Figure 10a,b: Enhancement of lipid transfection efficiency using SHE78-7 and RGE peptide.

Figure 11a,b: Enhancement of lipid transfection efficiency using SHE78-7 and RGD peptide.

30 Figure 12: Disruption of E-cadherin function comparing the antibody method with the calcium switch method.

Disruption of E-cadherin function with SHE78-7

5 The addition of the E-cadherin blocking antibody SHE78-7, caused efficient colony disruption in growing cultures (Fig. 1), with complete loss of all intercellular contacts in some areas. In addition, some cells showed reduced substrate adhesion, suggesting that the E-cadherin antibody might have had an indirect effect on integrin function.

10 The effect of SHE78-7 on confluent epithelia was studied. Epithelia grown on Transwell membranes that had attained transepithelial resistance (TER) of  $> 300\Omega \text{ cm}^2$  were exposed to SHE78-7 at 0.3-4 $\mu\text{g}/\text{ml}$  in the apical compartment. TERs were monitored as a measure of tight junction function. In 24h, the SHE78-7 antibody caused a concentration-related decline in TERs (Fig. 2). The highest concentration of SHE78-7 tested (4 $\mu\text{g}/\text{ml}$ ) resulted in a 90% fall in TER, indicating a loss of epithelial integrity.

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Adenoviral infection of 16HBE epithelia

20 To determine whether loss of epithelial integrity results in enhanced susceptibility to adenoviral infection, cultures were exposed to either control IgG2a or SHE78-7 for 24hrs, then infected with RAd35 for 6hrs. A dramatic increase in viral infection occurred in the SHE78-7 treated cultures (Fig. 3).

25 The dose response effect of the anti E-cadherin antibody on infection is shown in Fig. 4. The largest incremental increase in infection occurred between 1 and 4 $\mu\text{g}/\text{ml}$  SHE78-7, whilst the greatest change in TER occurred between 0.3 and 1.0  $\mu\text{g}/\text{ml}$  (Fig. 4).

Adenoviral infection of primary mouse tracheal epithelial cell cultures

30 Primary cultures of mouse tracheal epithelial cells were grown on Transwell COL membranes. These cultures were exposed for 24hrs to either control IgG or ECCD-2 and DECMA-1 antibodies then incubated with adenovirus for 6hrs. Cultures were then washed in PBS and extracted for measurement of  $\beta$ -galactosidase activity using a Promega  $\beta$ -galactosidase assay kit. (Fig 7).

Fig 6 shows the effect of anti-E-cadherin antibodies on transepithelial resistance. This experiment with primary mouse tracheal epithelial cells was analogous to the experiments with 16HBE14o- cells shown in Figs 2 and 3. The data show that 5 incubation of the mouse epithelial cells with the combination of the DECMA-1 and ECCD2 antibodies results in efficient disruption of the epithelial barrier as measured by a marked fall in TER (Fig 6). In addition, the epithelium treated with the E-cadherin antibodies was markedly more susceptible to RAd35 infection than control IgG treated cultures, where the epithelial barrier is maintained (Fig 10 7).

Effect of anti-integrin antibodies on transfection

SHE78-7 treated cultures were incubated with various anti-integrin antibodies for 15min prior to 6h viral exposure. Only the antibody against  $\alpha$  v  $\beta$ 5 integrin 15 inhibited SHE78-7 enhanced infection by up to 70% (Fig. 5).

Enhancement of lipid transfection using SHE78-7 antibodies

To DNA (CMVluc plasmid derived from pGL-3 Basic Vector) in OptiMEM buffer 20 was added DC-Chol, DOTAP or DMRIE-C lipid (DMRIE/cholesterol 1:1) and the mixture left for 15 mins at room temperature. The complexes were then put onto cells that had been treated with IgG2a control (4ug/ml) or SHE78-7 (4ug/ml) for 24 hours. Luciferase assays were performed after 24 hours.

Results are shown in Figs 8a and 8b and indicate that plasmid transfection 25 using DOTAP and DMRIE-C (Fig 8a) and DC-Chol and DOTAP (Fig 8b) were significantly enhanced in the presence of the SHE78-7 antibody compared with the transfection values obtained with the control antibody (IgG2a).

Effect of RGE peptide on adenoviral transfection

The experimental protocol was as in "Materials and Methods". Results are 30 shown in Figs 9a and 9b.

These figures demonstrate that the presence of the RGE peptide increases RAd35 infection in the presence of both the control antibody (Fig 9a) and the SH78-7 antibody (Fig 9b).

5 Effect of RGD and RGE peptide on lipid transfection

Peptide and DNA (CMVluc plasmid derived from pGL3 basic Vector) were mixed in OptiMEM buffer and left for 15 mins at room temperature. DOTAP, DC-Chol or EDMPC lipid was added and again left for 15 mins at room temperature. The complexes were then put onto cells that had been treated with IgG2a control 10 (4ug/ml) or SHE78-7 (4ug/ml) for 24 hours. Luciferase assays were performed after 24 hours.

Results are shown in Figs 10a, 10b, 11a and 11b.

These results indicate that the presence of the RGE peptide greatly enhances 15 the transfection efficiency of DOTAP, EDMPC and DC-Chol in combination with the anti E-cadherin antibody, SHE78-7, when compared with the results obtained for the control antibody, IgG2a (Figs 10a and 10b). The results shown in Figs 11a and 11b indicate that the RGD peptide has a similar effect upon lipid-mediated transfection as the RGE peptide.

20 Disruption of E-cadherin function comparing the antibody method with the calcium switch method.

Antibody method: Cultures were incubated for 24 h in Defined medium in both apical and basal compartments of Transwell membranes, the apical compartment also containing control IgG2a or SHE78-7 at 4 $\mu$ g/ml. After this 25 period, cultures were exposed to virus for 1h.

Ca<sup>2+</sup> switch method: Apical and basal culture media were removed, the cells washed 3 times with PBS and replaced with S-MEM containing 20 $\mu$ M CaCl<sub>2</sub>. Cultures were incubated in low Ca<sup>2+</sup> for 1 h, after which CaCl<sub>2</sub> was added to normalise the CaCl<sub>2</sub> concentration to 1.8mM and cells were exposed to virus for 30 1 h.

For both methods described above, after viral exposure, virus was removed from the wells and cultures were washed in PBS. Culture media was added and cells

were incubated for a further 5h. Cells were then washed and lysed and infection was assessed by measurement of  $\beta$ -galactosidase activity.

Lowering or depleting extracellular calcium is the most common method of disrupting cell-cell adhesion. However, the results in Fig. 12 show that 5 adenovirus mediated gene transfer is less efficient using this method compared with blocking E-cadherin function using the SHE78-7 antibody. The increased  $\beta$ -galactosidase activity in cultures treated with SHE78-7, compared to low  $\text{Ca}^{2+}$  treated cultures despite the comparable TER values both before and after 10 infection suggest that enhancement of infection by SHE78-7 engagement of E-cadherin is not simply due to a reduced epithelial barrier efficiency.

Example compositions

Example 1

15 A viral vector (e.g. adenoviral vector) expressing the therapeutic gene under control of a tissue-specific or constitutive promoter may be administered to patients together with an agent that disrupts adherens or tight junctions (e.g. an anti-E-cadherin antibody).

20 The virus and the E-cadherin or occludin inhibitor may be administered to the respiratory tract (e.g. by instillation or aerosolisation) or to tumours (e.g. by intratumoral or i.v. injection), to the liver (e.g. by i.v. injection).

25 The virus would be propagated in a complementing cell line that supports viral replication (e.g. HEK293, HER 911 or PerC6 cells). these cells may be grown in suspension cultures in bioreactors, as adherent cells in flasks or cell cubes, preferably in serum-free media. The virus would be collected 1-7 days after virus infection. The harvested virus may be purified by ultrafiltration, ion-exchange, affinity chromatography or differential centrifugation. and dialysed against physiological buffered saline. The virus would be stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ .

30

The anti-E-cadherin antibody would be produced by hybridoma cells and purified by ion exchange or affinity chromatography.

The virus and the inhibitor may be linked, injected together or sequentially into the same site or both targeted via addition of a targeting ligand (e.g. RGD peptide). Alternatively, RGE peptide may be administered together or before 5 administration of the virus to enhance the uptake of the virus.

The virus, E-cadherin or occludin inhibitor and the RGE peptides could be made up in physiological buffered saline (e.g. phosphate buffered saline or PBS).

10  $10^8$ - $10^{13}$  infectious virus particles may be administered with 0.1-10mg of anti-E-cadherin antibody with or without 0.1-10mg of RGE peptide.

**Example 2**

A non-viral traction vehicle consisting of a complex of DNA with a cationic lipid or polymer may be administered to patients together with an agent that disrupts 15 adherens or tight junctions (e.g. an anti-E-cadherin antibody).

The complex of DNA with a cationic lipid or polymer can be produced by mixing DNA (0.1-10mg/ml) with a lipid or polymer (1-20mg/ml) in water or buffer (e.g. 20 5mM Tris pH 7.5). RGD-polylysine (GGCRGDMFGCGG-(K)<sub>16</sub>) peptide or RGE-polylysine (GGCRGDMFGCGG-(K)<sub>16</sub>) peptide may be added (0.1-20mg/ml) to the DNA before or after adding the lipid to achieve targeting of the  $\alpha$  v integrins on the cell surface.

25 The DNA vector would encode the therapeutic gene for expression under control of a tissue-specific or constitutive promoter. The complex and the E-cadherin or occludin inhibitor may be administered to the respiratory tract (e.g. by instillation or aerosolisation) or to tumours (e.g. by intratumoral or i.v. injection), to the liver (e.g. by i.v. injection).

30 This complex may be diluted in a physiological buffer (e.g. phosphate buffered saline) before injection, instillation etc. The final concentration may be 0.1-10mg/ml and the injection volume may be 0.1-50 ml.

References, all of which are hereby incorporated into the text of this specification by reference:

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Claims

1. A pharmaceutical composition for gene therapy on cells which comprises:
  - 5 (a) nucleic acid encoding a therapeutic gene and a promoter;
  - (b) a transfection vehicle; and
  - (c) means to disrupt the function of the junctional complex in the cells.
- 10 2. A pharmaceutical composition according to claim 1 wherein the means to disrupt the function of the junctional complex in the cells is means to disrupt the adherens junction function.
- 15 3. A pharmaceutical composition according to claim 2 wherein the cells are epithelial cells and the means to disrupt the adherens junction function is means to disrupt E-cadherin function.
4. A pharmaceutical composition according to claim 3 wherein the means to disrupt E-cadherin function comprises an anti-E-cadherin antibody.
- 20 5. A pharmaceutical composition according to claim 2 wherein the cells are endothelial cells and the means to disrupt the adherens junction function is means to disrupt VE-cadherin function.
- 25 6. A pharmaceutical composition according to claim 5 wherein the means to disrupt the VE-cadherin function comprises an anti-VE-cadherin antibody.
7. A pharmaceutical composition according to claim 1 wherein the means to disrupt the function of the junctional complex in the cells is means to disrupt the tight junction function.

8. A pharmaceutical composition according to claim 7 wherein the means to disrupt the tight junction function is means to disrupt occludin function.
9. A pharmaceutical composition according to claim 5 wherein the means to disrupt occludin function comprises an anti-occludin antibody.
10. A pharmaceutical composition according to any one of claims 1 to 9 further comprising means to enhance cell surface integrin function or affinity of binding of the transfection vehicle to cell surface integrins.
11. A pharmaceutical composition according to any one of claims 1 to 10 wherein the transfection vehicle is a viral transfection vehicle.
12. A pharmaceutical composition according to claim 11 wherein the viral transfection vehicle is an adenoviral transfection vehicle.
13. A pharmaceutical composition according to claim 10 wherein the transfection vehicle is a viral transfection vehicle and the means to enhance cell surface integrin function or affinity of binding of the transfection vehicle to cell surface integrins comprises an RGE peptide.
14. A pharmaceutical composition according to claim 10 wherein the transfection vehicle is a viral transfection vehicle and the means to enhance cell surface integrin function or affinity of binding of the transfection vehicle to cell surface integrins comprises an RGD peptide which is bound to the viral transfection vehicle.
15. A pharmaceutical composition according to any one of claims 1 to 10 wherein the transfection vehicle is a non-viral transfection vehicle.

16. A pharmaceutical composition according to claim 15 wherein the non-viral transfection vehicle comprises a cationic lipid.
- 5 17. A pharmaceutical composition according to claim 10 wherein the transfection vehicle is a non-viral transfection vehicle and the means to enhance cell surface integrin function or affinity of binding of the transfection vehicle to cell surface integrins comprises an RGE peptide.
- 10 18. A pharmaceutical composition according to claim 10 wherein the transfection vehicle is a non-viral transfection vehicle and the means to enhance cell surface integrin function or affinity of binding of the transfection vehicle to cell surface integrins comprises an RGD peptide.
- 15 19. A pharmaceutical composition according to claim 17 or 18 wherein the RGD or RGE peptide has a cationic tail.
- 20 20. A method of enhancing efficiency of gene delivery to cells in a pharmaceutical composition for gene therapy comprising nucleic acid encoding a therapeutic gene and a promoter and a transfection vehicle which comprises including means to disrupt the function of the junctional complex in the cells.
- 25 21. A method of performing gene therapy on cells of a patient which comprises administering a composition which comprises:
  - (a) nucleic acid encoding a therapeutic gene and a promoter;
  - (b) a transfection vehicle; and
  - (c) means to disrupt function of the junctional complex of the cells.
- 30 22. Use of means to disrupt function of the junctional complex of cells together with nucleic acid encoding a therapeutic gene and a promoter and a transfection vehicle in the manufacture of a pharmaceutical composition for gene therapy.

23. A method of enhancing efficiency of gene delivery to cells in a pharmaceutical composition for gene therapy comprising nucleic acid encoding a therapeutic gene and a promoter, a viral transfection vehicle and means to disrupt function of the junctional complex in the cells which comprises including means to enhance cell surface integrin function or the affinity of binding of the viral transfection vehicle to cell surface integrins.

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24. A method of enhancing efficiency of gene delivery to cells in a pharmaceutical composition for gene therapy comprising nucleic acid encoding a therapeutic gene and a promoter, a non-viral transfection vehicle and means to disrupt the function of the junctional complex of the cells which comprises including means to enhance cell surface integrin function or affinity of binding of the non-viral delivery transfection vehicle to cell surface integrins.

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25. A method of performing gene therapy on cells of a patient which comprises administering a composition which comprises:

(a) nucleic acid encoding a therapeutic gene and a promoter;

(b) a viral transfection vehicle;

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(c) means to disrupt the function of the junctional complex of the cells; and

(d) means to enhance cell surface integrin function or affinity of binding of the viral transfection vehicle to cell surface integrins.

26. A method of performing gene therapy on cells of a patient which

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comprises administering a composition which comprises:

(a) nucleic acid encoding a therapeutic gene and a promoter;

(b) a non-viral transfection vehicle;

(c) means to disrupt the function of the junctional complex of the cells; and

30

(d) means to enhance cell surface integrin function or affinity of binding of the non-viral transfection vehicle to cell surface integrins.

27. Use of means to enhance cell surface integrin function or affinity of binding of a viral transfection vehicle to cell surface integrins together with nucleic acid encoding a therapeutic gene and a promoter, a viral transfection vehicle and means to disrupt the function of the junctional complex of cells in the manufacture of a pharmaceutical composition for gene therapy.

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28. Use of means to enhance cell surface integrin function or affinity of binding of a non-viral transfection vehicle to cell surface integrins together with nucleic acid encoding a therapeutic gene and a promoter, a non-viral transfection and means to disrupt the function of the junctional complex of cells in the manufacture of a pharmaceutical composition for gene therapy.

10

29. A pharmaceutical composition for gene therapy which comprises:

15 (a) nucleic acid encoding a therapeutic gene and a promoter;

(b) a viral transfection vehicle; and

(c) means to enhance cell surface integrin function or the affinity of binding of the viral transfection vehicle to cell surface integrins.

30. A pharmaceutical composition for gene therapy which comprises:

20 (a) nucleic acid encoding a therapeutic gene and a promoter;

(b) a non-viral transfection vehicle; and

(c) means to enhance cell surface integrin function or the affinity of binding of the non-viral transfection vehicle to cell surface integrins.

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Peptide sequence of human E-cadherin (extracellular domain is underlined and bold):

**MGPWSRSLSALLLLQVSSWLCQEPEPCHPGFDAESYTFTVPRRH**  
 LERGRVLGRVN **FEDCTGRQRTAYFSLDTRFKVGTG** VITVKRPLRFHN **PQIHF** LVYAWD  
 STYRKFSTKVTLNTVGHHRRPPHQASVSGIQAELLTPNSSPG **LLRQKRDWV** IPPISC  
 PENEKGPFPKNLVQI **KSNDKEGKV** FYSITGQGADTPPVGVF **IIE** RETGWLKVTEPLDR  
**ERIATYTLF** SHAVSSNGNAVEDPME **ILIT** VTDTQDN **DKPEFTQEVFKGSVME** GALPGTSV  
 MEVTATDADDDVNTYNA **ALAY** TILSQDPELPDKNMFTINRNTGVISVVTG **LDRE** SFPT  
 YTLVVQO **ADLQ** GEGLSTTATAVITVTDNDN **PPIFNPT** TYKGQV **PENEANVV** ITTLKVT  
 DADAPNTPAWEAVY **TI** LNDGGFVVTTNPVNDGILKTA **GLDFEAKQ** QYILHVAVTN  
 VVPFEVSLTTSTATVTDVLDVNEAP **IFV** PPEKRVEVSEDFVGQ **QEITSY** TAQEPDTFM  
 EOKITYRIWRDTANWLE **INPD** TGA **ISTRAEL** DREDFEHVKNSTY **TALI** IATDNGSPVAT  
 GTGT **LL** LLSVNDNAPIPEPRTIFFCERNPKPQVINI **I** DADLPPNTSPFTAELTHGAS  
 ANWTIQYNDPTQESI **IL** KPKMALEVGDYKINLKLMDNQNKDQVTTLEVSVCDC **EGAAGV**  
 CRKAQFVEAGLQI **PAIL** GILGGI **ALLL** LLLLFLRRRAVVK **EPLL** P **PEDD** TRDNVY  
 YYDEEGG **GEEDQDF** DLSQLHRLDARPEVTRNDVAP **TLMSV** PRYLPRPANPDEIGNFID  
 ENLKAADTDPTAPPYDSLLVFDYEGSGSEAASLSSLNSSES **DKDQDY** DYLNEWGNRFKK  
 LADMYGGEDD.

Nucleotide sequence encoding human E-cadherin:

GACTGGTTATTCCCTCCATCAGCTGCCAGAAAATGAAAAAGGCCATTCTCTAAAAAC  
 CTGGTCAGATCAAATCCAACAAAGACAAGAGCAAGGCTTCTACAGCATCACTGGC  
 CAAGGAGCTGACACACCCCCCTGTTGGTCTTTATTATTGAAAGAGAACAGGATGGCTG  
 AAGGTGACAGAGCCTCTGGATAGAGAACGCATGCCACATACACTCTCTCTCACGCT  
 GTGTCATCCAACGGGAATGCAGTTGAGGATCCAATGGAGATTTGATCACGGTAACCGAT  
 CAGAATGACAACAAGCCGAATTCACCCAGGAGGTCTTAAGGGCTGTCTGGAAAGGT  
 GCTCTCCAGGAACCTCTGTGATGGAGGTACAGGCCACAGCGGACGATGATGTGAAC  
 ACCTACAATGCCCCATCGCTTACACCACCTCTCAGCAAGATCCTGAGCTCCCTGACAAA  
 AATATGTTACCATTAAACAGGAACACAGGAGTCATCAGTGTGGTCAACACTGGCTGGAC  
 CGAGAGAGTTCCCTACGTATACCCGGTGGTCAAGCTGCTGACCTCAAGGTGAGGGG  
 TTAAGCACAACAGCAACAGCTGTGATCACAGTCAGTACACCAACGATAATCTCCGATC  
 TTCAATCCCACCACTCGTACAAGGGTCAGGTGCCTGAGAACGAGGCTAACGTCGTAATCACC  
 AACTGAAAGTGA **CTGATG** TGTGATGCC **CCAA** TACCCAGCGTGGAGGCTGTATACACC  
 ATATTGAATGATGATGGTGGACAATTGTCGTCAAC **CAAATCC** AGTGAACAACGATGGC  
 ATTTGAAAACAGCAAAGGGCTTGGATTTGAGGCCAGCAGCAGTACATTCTACAGTA  
 GCAGTGACGAATGTGGTACCTTTGAGGTCTCTCACCCACGCCACCGTCACC  
 GTGGATGTGCTGGATGTGAATGAAGCCCCATCTTGTCCTCTGAAAGAGAGTGAA  
 GTGTCCGAGGACTTGGCTGGCCAGGAATCACATCCTACACTGCCAGGAGCCAGAC  
 ACATTATGGAACAGAAAATAACATATCGGATTGGAGAGACACTGCCA **ACTGGCTGGAG**  
 ATTAATCCGGACACTGGTGCCTTCACTCGGGCTGAGCTGGACAGGGAGGATTTGAG  
 CACGTGAAGAACAGCACGTACACAGCCCTAATCATAGCTACAGACAATGGTCTCCAGTT  
 GCTACTGGAACAGGGACACTCTGCTGATCCTGCTGATGTGAATGACAACGCC **CATA**  
 CCAGAACCTCGAACTATATTCTCTGTGAGAGGAATCCAAAGCCTCAGGTCAAAACATC  
 ATTGATGCAGACCTTCCCTCCAAATACATCTCCCTCACAGCAGAACTAACACACGGGGCG  
 AGTGCCA **ACTGG** ACCATTCACTGACAAACGACCCAAAGAACATCTATCATTTGAAGCCA  
 AAGATGGCCTTAGAGGTGGGTGACTACAAAATCAATCTCAAGCTCATGGATAACCAGAAAT  
 AAAGACCAAGTGACCACCTTAGAGGTCA **CAGCGTGTGACTGT** GAAGGGCCGCCGGCGTC  
 TGTAGGAAGGCACAGCCTGTCGAAGCAGGATTGCAAATT

**FIG. A**

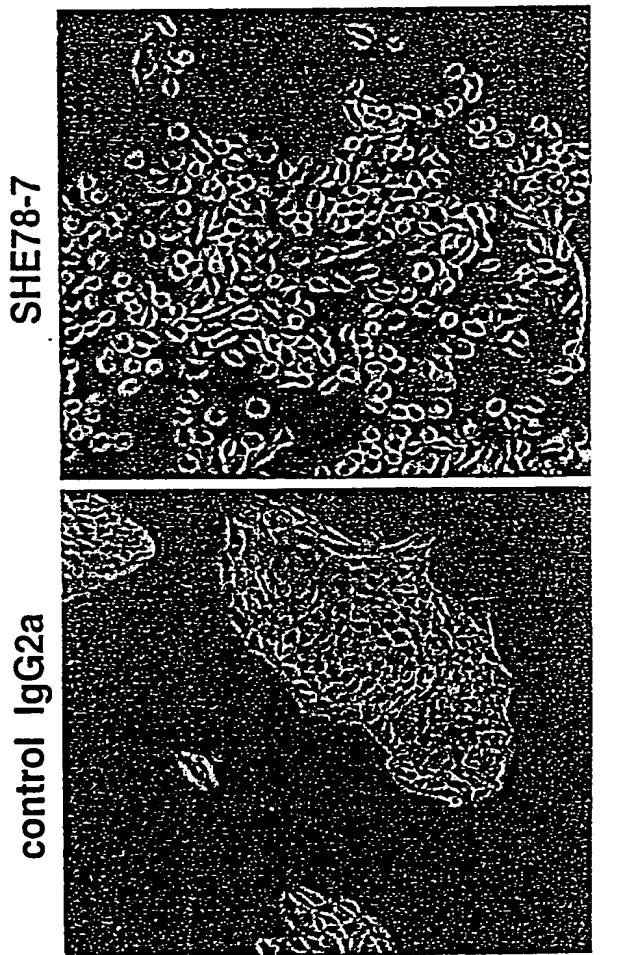
## FIG. B

Peptide sequence of human occludin:

MSSRPLESPPPYRPDEFKPNHYAPSNDIYGGEMIHPMLSQPAYSFYPEDIELHFYKWT  
PPGVIRILSMLIIVMCAIFACVASTLAWDRGYGTSSLGGSGFGSYGSYGGYGGYGGY  
TDPRAAKGFMILAMAACFCIAALVIFVTSVIRSEMSTRRYYLSVIVSAILGIMVFIATIVYIMGVNPTAQSSGS  
LYGSQIYALCNQFYTPAAATGLYVDQYLHYCWDQPLQEAIIAVLGFMIIIVAFALIIFFAVKTRRKMDRYDKSNIL  
WDKEHIYDEQPPNVEEWVKNSAGTQDVPSPPSDYVERVDSKRTEQDHYETDYGESCDELEEDWIR  
WQELPLTSPVDDFRQPRYSSGGNFETPSKRAPAKGRAGRSKRTEQDHYETDYGESCDELEEDWIR  
EYPPITSDDQQRQLYKRNFDTGLQEYKSLQSELDEINKELSRLDKEELDDYREESEEYMAADEYNRLKQVK  
GSADYKSKKNNHCKQLSKLSHIIKKMVGDYDRQKT

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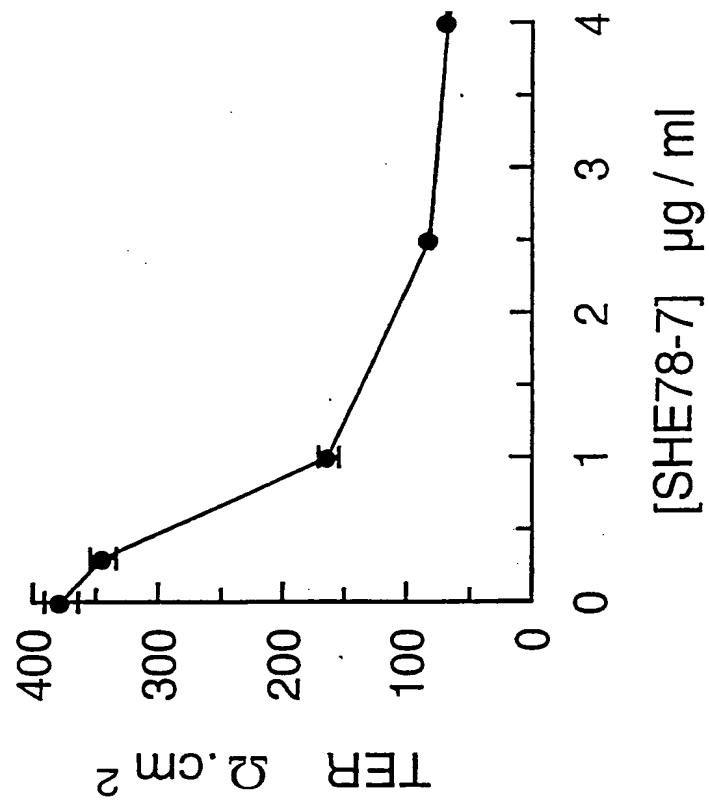
FIG. 1  
16HBE14o- cells incubated with control IgG2a  
or SHE78-7 for 24hrs



[Antibody] : 3 $\mu$ g/ml

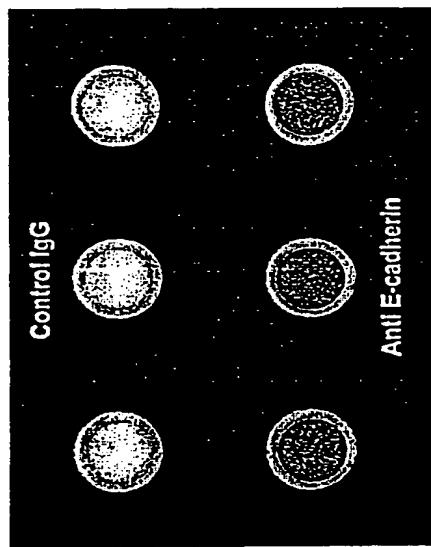
4/18

**FIG. 2**  
Effect of SHE78-7 on transepithelial resistance of 16HBE14o- cells



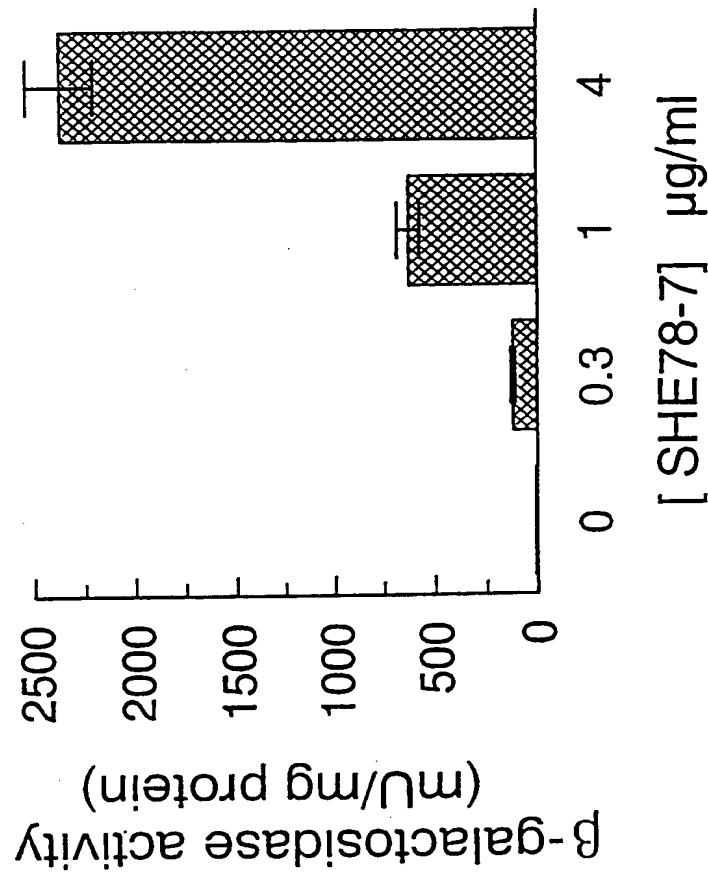
5 / 18

FIG. 3  
16HBE140- cells incubated with IgG2a or SHE78-7 antibody for 24hrs



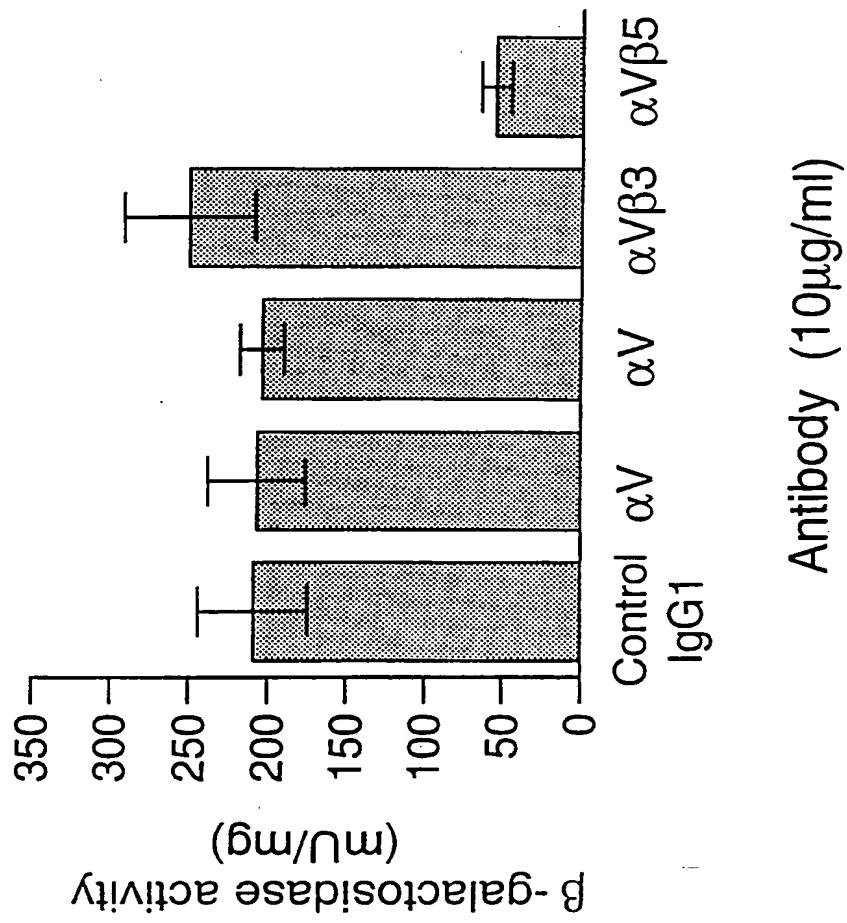
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FIG. 4  
Effect of SHE78-7 on RAd35 infectivity of 16HBE140- cells



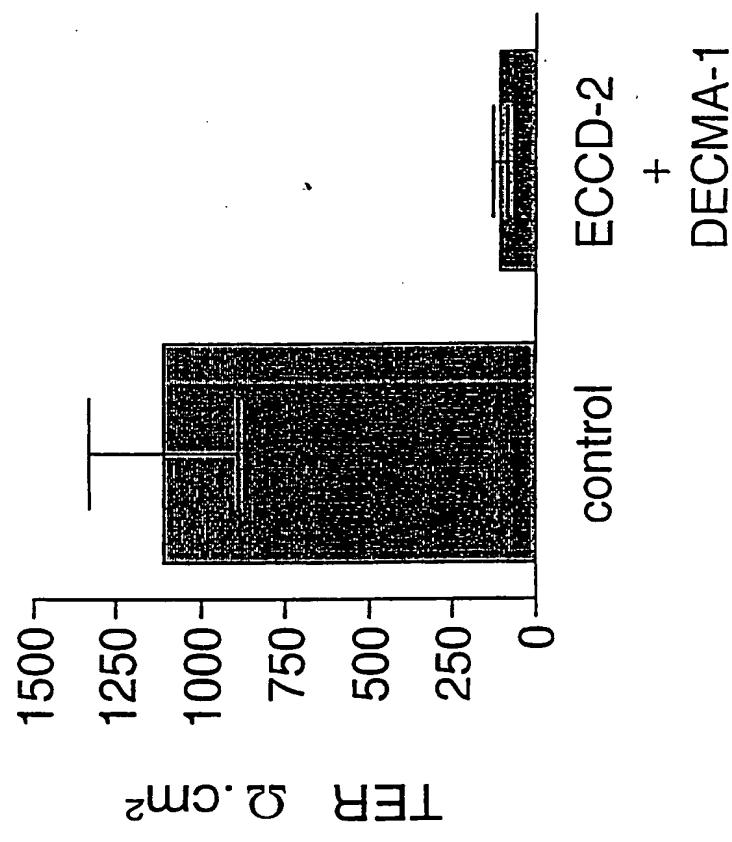
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FIG. 5  
Effect of anti-integrin antibodies on RAd35  
infectivity of SHE78-7 treated 16HBE140- cells



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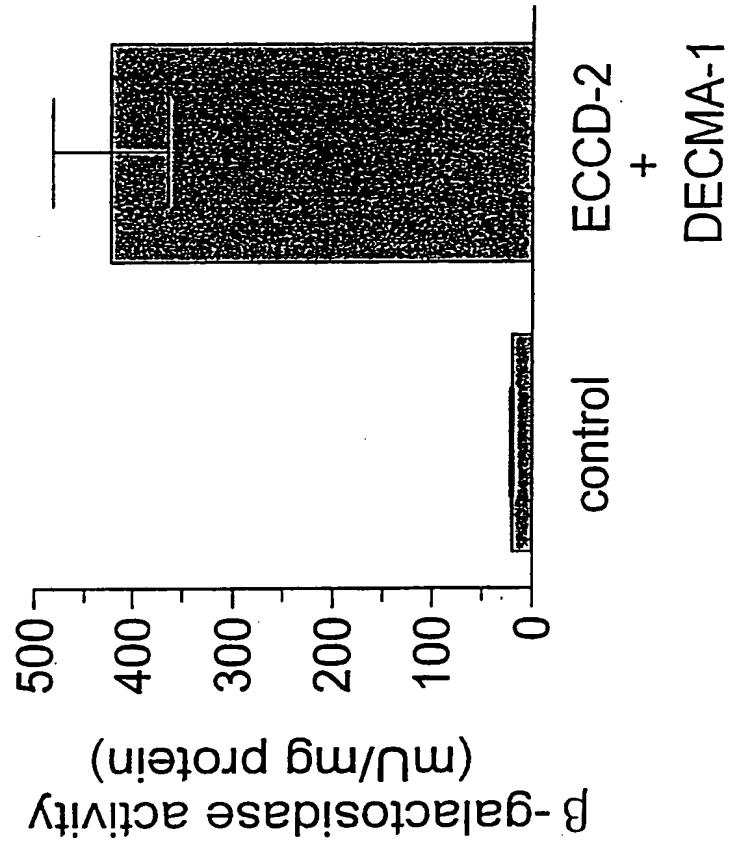
**FIG. 6**  
**Effect of anti E-cadherin antibodies on TER of**  
**mouse primary tracheal epithelial cells**



ECCD-2 and DECMA-1 were tested at 10 $\mu$ g/ml

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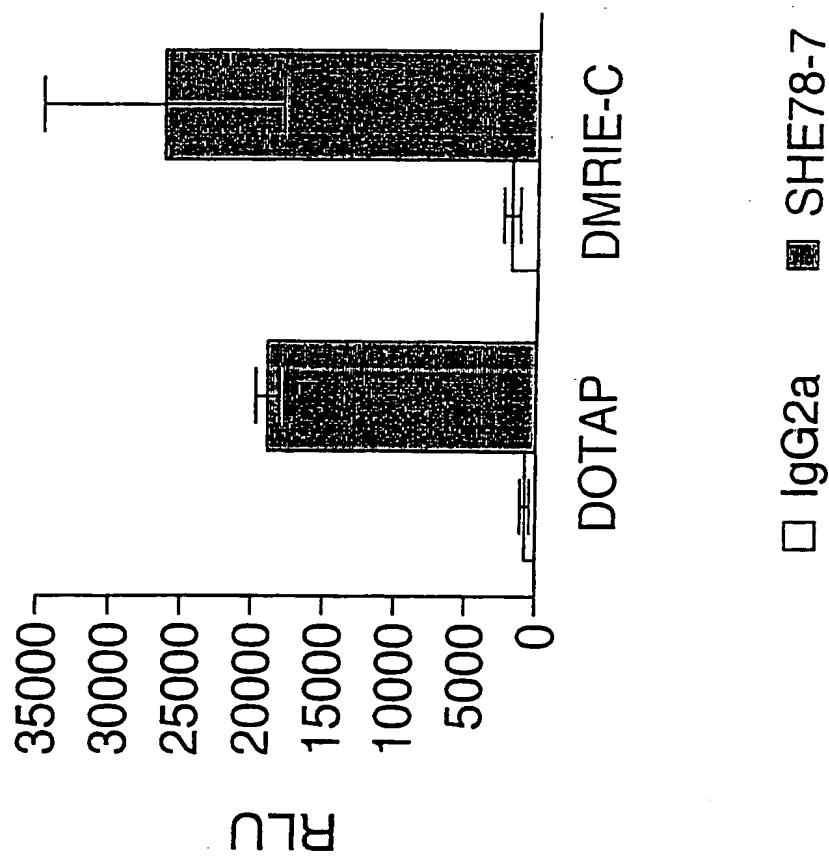
**FIG. 7**  
Effect of anti E-cadherin antibodies on RAd35 infectivity of mouse primary tracheal epithelial cells



ECCD-2 and DECMA-1 were tested at 10 $\mu$ g/ml

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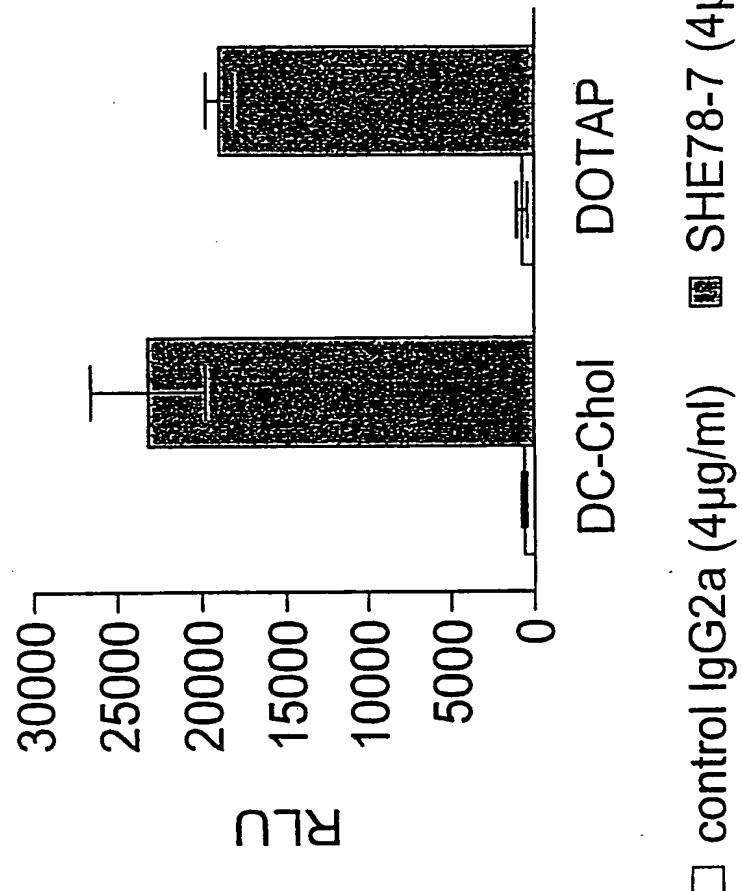
**FIG. 8a**  
**Enhancement of lipid mediated gene delivery to 16HBE140-  
cells using anti E-cadherin antibody**



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**FIG. 8b**

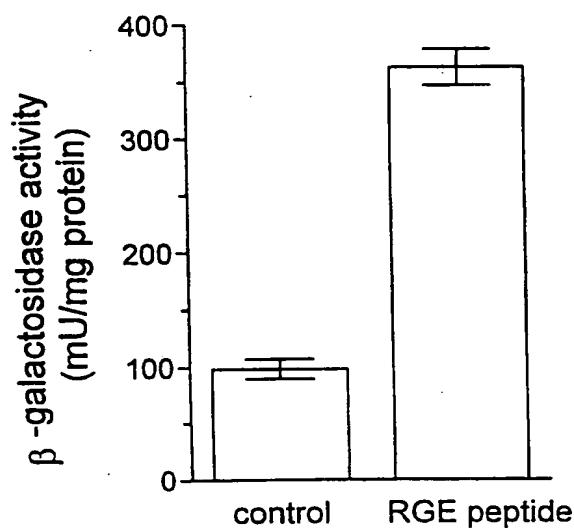
**Enhancement of lipid mediated gene delivery to 16HBE14o- cells using anti E-cadherin antibody**



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## FIG. 9a

The effect of RGE peptide on RAd35 infection of control  
IgG2a treated 16HBE14o- cells

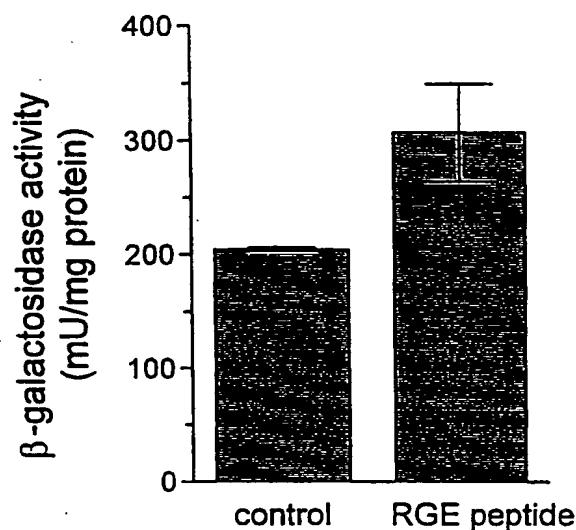


Cells were treated with 4 $\mu$ g/ml of control IgG2a antibody.  
RGE peptide (GRGESP) was tested at 0.4mg/ml.  
control: cell culture media

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**FIG. 9b**

The effect of RGE peptide on RAd35 infection of SHE78-7  
treated 16HBE14o- cells

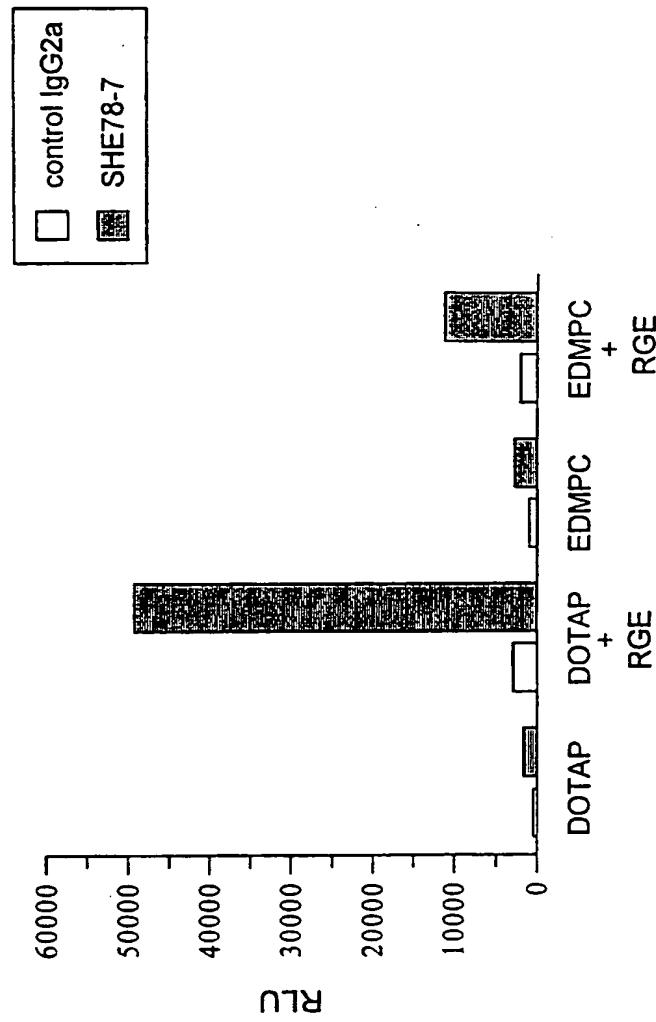


Cells were treated with 4 $\mu$ g/ml of SHE78-7 antibody.  
RGE peptide (GRGESP) was tested at 0.2mg/ml.  
control: cell culture media

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FIG. 10a

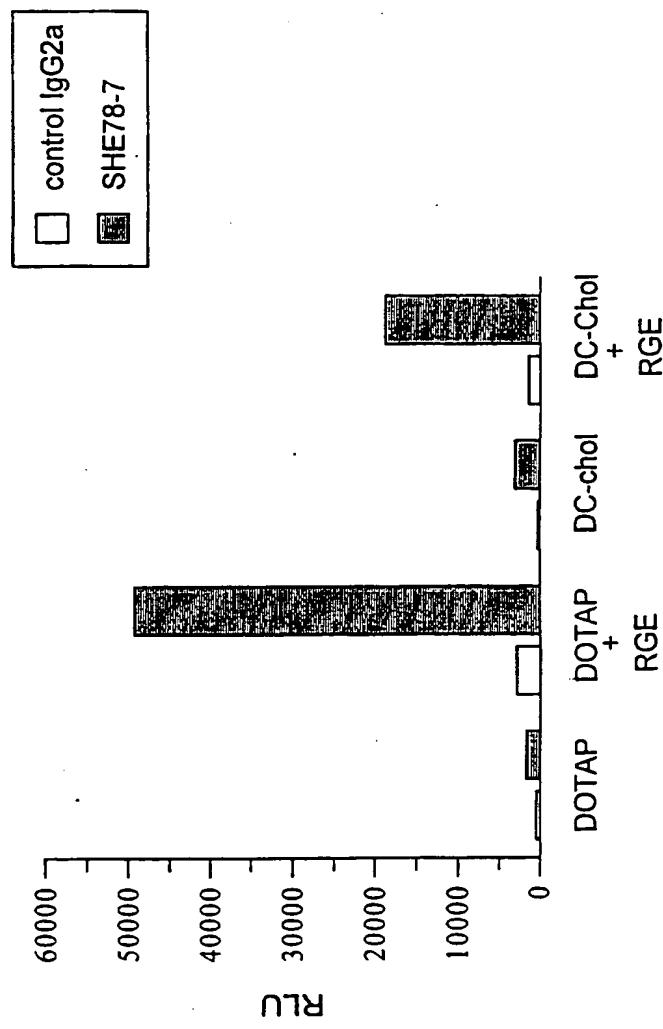
Enhancement of lipid transfection efficiency using anti  
E-cadherin antibody and RGE peptide



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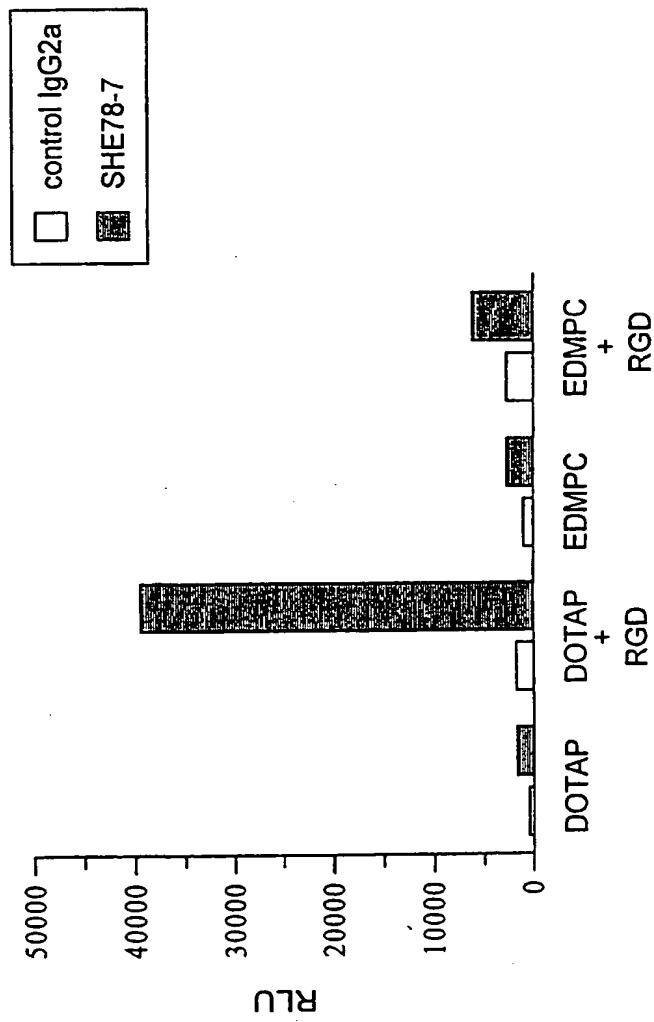
FIG. 10b

Enhancement of lipid transfection efficiency using anti  
E-cadherin antibody and RGE peptide



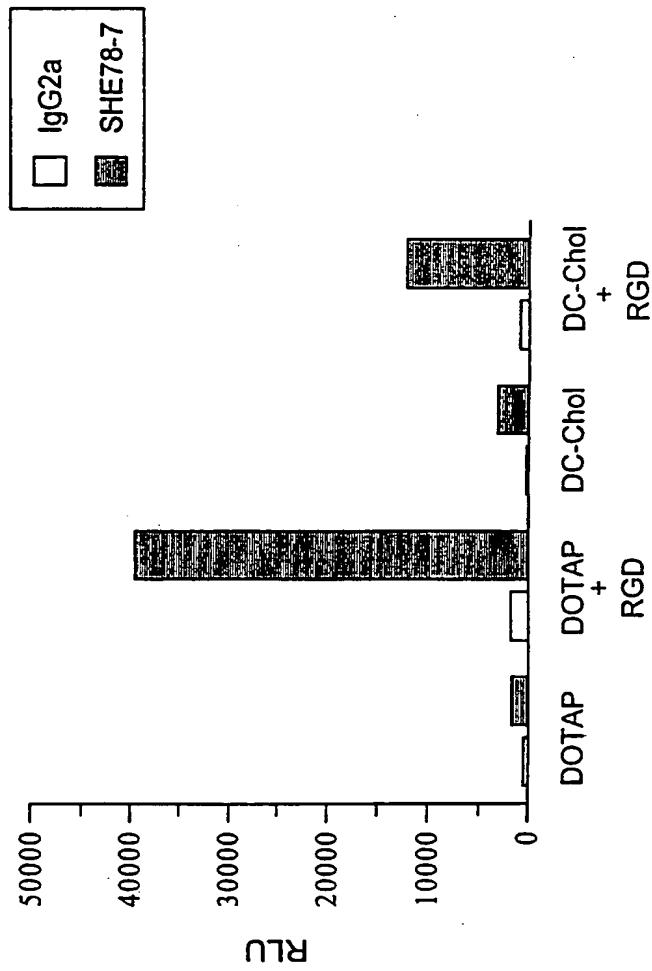
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**FIG. 11a**  
Enhancement of lipid transfection efficiency using  
anti E-cadherin antibody and RGD peptide



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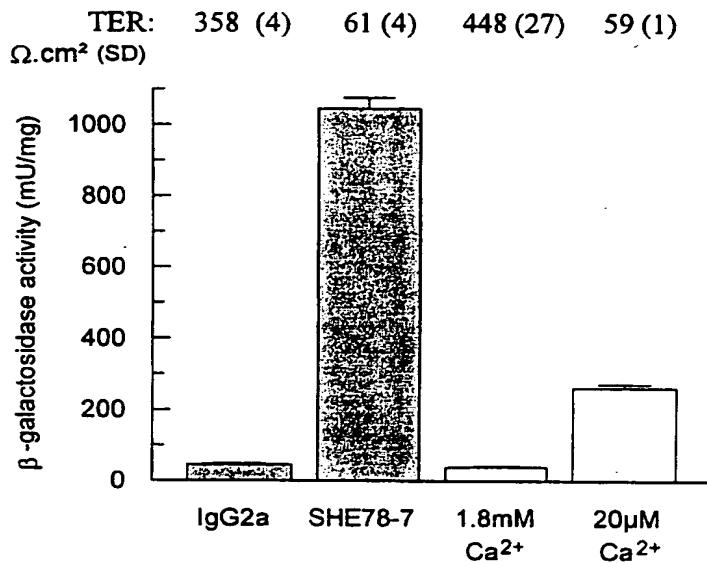
**FIG. 11b**  
Enhancement of lipid transfection efficiency using  
anti E-cadherin antibody and RGD peptide



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**FIG. 12**

Disruption of E-cadherin function comparing the antibody method with the calcium switch method



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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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WO 00/62815 A3

(54) Title: NOVEL PHARMACEUTICAL COMPOSITION SUITABLE FOR GENE THERAPY

(57) Abstract: There is provided according to the invention a pharmaceutical composition for gene therapy on cells which comprises: (a) nucleic acid encoding a therapeutic gene and a promoter; (b) a transfection vehicle; and (c) means to disrupt the function of the junctional complex in the cells together with methods of treatment using the same.

# INTERNATIONAL SEARCH REPORT

Int. Application No  
PCT/GB 00/01408

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, BIOSIS, MEDLINE, CHEM ABS Data, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	page 443, left-hand column, paragraph 3 -page 445, left-hand column, paragraph 1 ---	3-6,8,9
X	CHU Q ET AL: "Binding and uptake of cationic lipid:pDNA complexes by polarized airway epithelial cells." HUMAN GENE THERAPY, vol. 10, no. 1, 1 January 1999 (1999-01-01), pages 25-36, XP000952585 ISSN: 1043-0342 cited in the application the whole document ---	1,2,7, 10-12, 14-16, 18-28
Y	---	3-6,8,9
		-/-

Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

17 October 2000

Date of mailing of the international search report

26/10/2000

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**INTERNATIONAL SEARCH REPORT**

International Application No  
PCT/GB 00/01408

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 97 33605 A (ITALLIE CHRISTINA M VAN ;UNIV YALE (US); ANDERSON JAMES M (US)) 18 September 1997 (1997-09-18) abstract page 10, line 18 - line 22 ---	1,2, 7-12, 14-16, 18-28
A	HARBOTTLE R P ET AL: "An RGD -oligolysine peptide: a prototype construct for integrin-mediated gene delivery 'see comments!'" HUMAN GENE THERAPY,XX,XX, XP002080440 ISSN: 1043-0342 the whole document ---	10,13, 14, 17-19, 23-28
X		29,30
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X		29,30
A	WICKHAM T J ET AL: "Increased in vitro and in vivo gene transfer by adenovirus vectors containing chimeric fiber proteins" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 11, no. 71, 1 November 1997 (1997-11-01), pages 8221-8229, XP002078898 ISSN: 0022-538X cited in the application the whole document ---	10,14, 18,19, 23-28
X		29,30
Y	WO 91 04745 A (ATHENA NEUROSCIENCES INC) 18 April 1991 (1991-04-18) the whole document ---	3-6

**INTERNATIONAL SEARCH REPORT**

International Application No  
PCT/GB 00/01408

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	KOTZE A F ET AL: "Comparison of the effect of different chitosan salts and N-trimethyl chitosan chloride on the permeability of intestinal epithelial cells (Caco-2)" JOURNAL OF CONTROLLED RELEASE, NL, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, vol. 51, no. 1, 23 January 1998 (1998-01-23), pages 35-46, XP004108275 ISSN: 0168-3659 the whole document ---	1-28
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**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-28

pharmaceutical composition for gene therapy which comprises  
: (a) nucleic acid encoding a therapeutic gene and a promoter  
    (b) a transfection vehicle  
    (c) means to disrupt the function of the junctional complex  
        in the cells

2. Claims: 29-30

pharmaceutical composition for gene therapy which comprises  
: (a) nucleic acid encoding a therapeutic gene and a promoter  
    (b) a transfection vehicle  
    (c) means to enhance cell surface integrin function or the  
        affinity of binding of the transfection vehicle to cell  
        surface integrins

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/GB 00/01408

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
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CA 2271826	A	NONE			